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Bacterial Cell Surface Display of an Enzyme Library for Selective Screening of Improved Cellulase Variants

Yong-Sung Kim, Heung-Chae Jung, and Jae-Gu Pan*

Bioprocess Engineering Division, Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon 305-600, Korea

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The bacterial surface display method was used to selectively screen for improved variants of carboxymethyl cellulase (CMCase). A library of mutated CMCase genes generated by DNA shuffling was fused to the ice nucleation protein (Inp) gene so that the resulting fusion proteins would be displayed on the bacterial cell surface. Some cells displaying mutant proteins grew more rapidly on carboxymethyl cellulose plates than controls, forming heterogeneous colonies. In contrast, cells displaying the nonmutated parent CMCase formed uniform tiny colonies. These variations in growth rate were assumed to result from altered availability of glucose caused by differences in the activity of variant CMCases at the cell surface. Staining assays indicate that large, rapidly growing colonies have increased CMCase activity. Increased CMCase activity was confirmed by assaying the specific activities of cell extracts after the expression of unfused forms of the variant genes in the cytoplasm. The best-evolved CMCases showed about a 5- and 2.2-fold increase in activity in the fused and free forms, respectively. Sequencing of nine evolved CMCase variant genes showed that most amino acid substitutions occurred within the catalytic domain of the enzyme. These results demonstrate that the bacterial surface display of enzyme libraries provides a direct way to correlate evolved enzyme activity with cell growth rates. This technique will provide a useful technology platform for directed evolution and high-throughput screening of industrial enzymes, including hydrolases.

* Corresponding author. Mailing address: Bioprocess Engineering Division, Korea Research Institute of Bioscience and Biotechnology (KRIBB), P.O.B. 115, Yusong, Taejon 305-600, Korea. Phone: 82-42-860-4483. Fax: 82-42-860-4594. E-mail: jgpan@kribb4680.kribb.re.kr .

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